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THE EFFECTS OF 6-MONTH CHRONIC LOW LEVEL INHALATION EXPOSURES TO HYDRAZINE ON ANIMALS

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Major technical areas discussed included ecosystem modeling, water pollution, water reuse, toxic hazards evaluation of fire extinguishants, environmental carcinogenesis, and cellular toxicology.

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PAPER NO. 16

THE EFFECTS OF 6-MONTH CHRONIC LOW LEVEL INHALATION EXPOSURES TO HYDRAZINE ON ANIMALS

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Hydrazine (N_2H_4) is a highly reactive reducing agent which is widely used as an intermediate in organic synthesis and either singly or in combination with other hydrazines such as 1,1-dimethylhydrazine or methylhydrazine as a missile propellant. It is also used extensively as a corrosion inhibitor in boiler feed water. Hydrazine is a colorless liquid with a molecular weight of 32.05, density of 1.008 g/ml and a vapor pressure of 14.4 mm Hg at 25 C.

Hydrazine is a strong convulsant at high doses but may cause central nervous system depression at lower doses. Its toxicity and pharmacologic effects are detailed in a comprehensive review by Clark et al. (1968). Animals may die acutely of convulsions, respiratory arrest, or cardiovascular collapse within a few hours of an acute exposure by any route of administration, or may die 2 to 4 days later of liver and kidney toxicity (Weir et al., 1964; Witk'n, 1956). Jacobson et al. (1955) reported the 4-hour inhalation LC_{50} value as 252 ppm (330 mg/m^3) for the mouse and 570 ppm (750 mg/m^3) for the rat.

House (1964) exposed monkeys, rats and mice to a hydrazine concentration of 1.0 ppm continuously for 90 days. Though mortality was very high, some animals survived the experiment. Ninety-six percent of the rats and 98% of the mice died during the exposure while monkeys proved to be the most resistant species with only a 20% mortality.

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Comstock et al. (1954) exposed dogs, in separate experiments, to 5 and 14 ppm. Two dogs survived repeated 6-hour exposures to 5 ppm hydrazine for 6 months and 2 of 4 dogs lived after 194 six-hour exposures to 14 ppm; the other two dogs died during the third and fifteenth weeks in a debilitated condition. The dog that died during the fifteenth week had a severe convulsive seizure prior to death. Prior to death, both dogs showed signs of anorexia and general fatigue. Changing diets and forced feedings resulted in the survival of the remaining two dogs.

The present Threshold Limit Value (TLV) published by the American Conference of Governmental Industrial Hygienists (1973) for N_2H_4 is 1 ppm or 1.3 mg/m^3.

(U) To compare the effects of repeated ~~6 hour per day, 5 day per week~~ (industrial type) exposures with continuous exposures of equivalent concentrations and to evaluate the safety factor of the current TLV, four concentration levels were selected for the 26-week exposure of four animal species. The concentrations selected were 1.0 ppm and 0.2 ppm for continuous exposures and 5.0 ppm and 1.0 ppm for intermittent daily exposures. These concentrations would result in the following CT (concentration x time) values:

1.0 ppm continuous	=	168 ppm-hours per week
5.0 ppm intermittent	=	150 ppm-hours per week
1.0 ppm intermittent	=	30 ppm-hours per week
0.2 ppm continuous	=	33.6 ppm-hours per week.

Thus, the 1.0 ppm continuous and the 5.0 ppm intermittent studies would be relatively equivalent doses and the 1.0 ppm intermittent and 0.2 ppm continuous would also be comparable.

Four exposed groups and a control group were used in these experiments. Each consisted initially of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats and 40 female CF-1 mice. The animals were monitored throughout the 6 months of exposure with biological measurements made at biweekly intervals. These measurements consisted of hematology and clinical chemistry values, body weights, physical examinations, and on selected animals bone marrow examinations were conducted. The details of the experimental methodology and findings were presented by Haun and Kinkead (1973) with the exception of the results in groups of rats and mice held for long-term postexposure observation. Ten rats and 10 mice from each experimental and control group were set aside at the end of the 6-month exposure period and maintained in an animal holding room for long-term postexposure observation.

(U) The effects of chronic inhalation of hydrazine are dose related regardless of the nature of exposure, i.e., intermittent or continuous. The highest hydrazine dose caused approximately 40% deaths in mice within the first two months of exposure while the TLV dose equivalents caused approximately 5% mortality.

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Although mice were not weighed, rats exhibited a dose related growth rate depression and dogs exposed to hydrazine showed weight loss at the highest dose levels.

The most significant or noticeable signs of stress occurred in the case of the dogs exposed to 1 ppm continuously. Weight loss was very noticeable ~~in these dogs, and although we did not measure~~ and food consumption ~~it~~ was ~~obviously~~ reduced. Anorexia continued with progressive emaciation until about 16 weeks when some recovery occurred in the surviving dogs. One dog in this group experienced toxic convulsions on 3 separate occasions, once after 3 months of exposure, then once in the morning and once in the afternoon of the same day after 5 months of exposure. These findings were consistent with those reported by Comstock et al. (1954).

(u) In animals held postexposure, weight differences between control and exposure groups became insignificant by four weeks.

There were no abnormal findings in clinical chemistry and hematology measurements made on monkeys and rats. Dogs, however, had a hydrazine dose related depression of red blood cell counts, hemoglobin values, hematocrits, and there was little or no reticulocytosis before the fifth month of exposure at which time the dogs continuously exposed to 1 ppm N_2H_4 had a sharp depression of RBC count accompanied by reticulocytosis. At necropsy, this group of dogs was the only group of any species to demonstrate erythropoietic activity as measured by a decreased myeloid erythroid ratio in bone marrow.

There was no measurable evidence of red blood cell destruction in these dogs exposed to hydrazine in contrast with readily demonstrated hemolytic activity of monomethylhydrazine (MMH) (MacEwen and Haun, 1971). Furthermore, the red blood cells of dogs exposed to N_2H_4 were markedly more susceptible to osmotic fragility than control animals while MMH produced a significant increase in RBC fragility. We were unable to determine the precise reason for the hydrazine induced anemia or to explain the decreased RBC fragility during these experiments but plan to explore this area further.

(u) The results of gross and histopathologic examination of mice that died during exposure showed that death was due to hydrazine hepatotoxicity. At sacrifice, moderate to severe fatty liver change was a consistent finding in mice from all exposure levels. Monkey livers showed slight to moderate fat accumulation. Perhaps compromising part of this information is the fact that control monkeys also showed some degree of fatty liver change. Malnutrition, the result of nonspecific hydrazine toxicity, caused the death of 2 dogs in the 1 ppm continuous exposure. At sacrifice, dogs exposed to the TLV concentration showed no abnormalities but dogs from the high doses had fatty livers. Since one dog in the 1 ppm continuous exposure group convulsed during exposure, the brains of this dog and 3 others in the same group were perfused at

sacrifice. Histology revealed no CNS lesions. Two dogs each from the high concentration experiments were sacrificed at 6 weeks postexposure. All were described as being essentially normal animals.

Organ weights of exposed rats, monkeys and dogs were not statistically different from control values. In the case of the rats, the depressed growth rates resulted in increased organ to body weight ratios to which no biological significance can be attributed.

There were no significant pathologic changes in rats except in the case of the 5 ppm intermittent exposure group. Of the 30 rats, 19 had chronic bronchopneumonia. Whether this condition was due to a hydrazine pulmonary irritation or pathogens present, or the former predisposing the rats to the latter, is difficult to say. The net effect, however, was that 10 rats from this group retained postexposure showed no weight recovery as demonstrated by the other exposed groups. The infection spread to other rat groups housed in the same room and within 6-8 weeks following exposure termination, 50% of the rats were dead. The number of deaths was distributed rather evenly in the exposed groups and in the controls as well. Consequently, none of the rats survived long enough for conclusions to be drawn about the carcinogenic potential of inhaled hydrazine for this species.

Tumorigenesis has, however, been demonstrated in rats following daily oral administration of 12 or 18 mg hydrazine sulfate doses over a 68 week period. Pulmonary adenocarcinomas, hepatic cell and spindle cell carcinomas were observed after 109 weeks (Severi and Bianchiori, 1968). They found no lung or liver tumors in their untreated control rats.

Approximately half the mice in each group were alive 1 year postexposure. At necropsy, non-neoplastic lesions were found in the ICR/CF-1 mice with approximately equal frequency in both experimental and control groups. An occasional mouse had a mammary gland adenoma, but since these are normally found with an incidence of 5-10% in mice, they were considered to be unrelated to the hydrazine exposure. For similar reasons, a single small papilloma found in one exposed mouse was not considered significant.

Some of the mice exposed to the threshold limit value concentration of hydrazine (1 ppm) had well differentiated alveolargenic carcinomas as shown in Figure 1. Another of these tumors (Figure 2) shows invasion of the pleura and extension into the pleural space. In mice of the 5 ppm intermittent exposure group, alveolargenic carcinomas were also seen. These tumors had a greater frequency of metastatic activity with tumors found in liver and in the ribs as shown in Figures 3 and 4. Many mice exposed to 1 ppm hydrazine on a continuous basis developed alveolargenic carcinomas. One had a hepatoma as shown in Figure 5. There was one rather poorly circumscribed area of the liver in which the cells were large with variable sized nuclei, many of

which contained a large eosinophilic intranuclear inclusion. In this same animal most of the spleen was replaced by neoplastic tissue (Figure 6). Most of this tissue was very anaplastic as shown in Figure 7, but in a few areas the cells resembled neoplastic hepatocytes. Two mice in this group developed lymphosarcoma in the spleen (Figure 8) which was extremely cellular with a loss of normal architecture. The cells are uniformly immature lymphocytes with numerous mitotic figures. There is invasion of the capsule with a great deal of phagocytosis by macrophages. This, in combination with the other morphology, is diagnostic of lymphosarcoma and is substantiated by an adjacent lymph node (Figure 9) which shows complete loss of architecture due to these neoplastic lymphoid cells. Similar changes were also seen in liver, kidney, lung and the urinary bladder.

The tumor incidences shown in Table I are believed significant for two reasons. First, alveolargenic carcinomas are found in higher, dose related, frequencies among exposed mice than in controls. Second, lymphosarcoma and the uncommon malignant hepatoma are absent from controls but occur in mice exposed to the higher dose.

TABLE I. TUMOR INCIDENCE IN MICE ONE YEAR AFTER CHRONIC INHALATION EXPOSURE TO HYDRAZINE (6-MONTH EXPOSURE PERIOD)

Exposure	Alveolargenic Carcinoma	Lympho-sarcoma	Hepatoma	Number of Mice with Tumors
High Dose				
1.0 ppm Continuous	5/9	2/9	1/9	6/9
5.0 ppm Intermittent	5/6	0/6	0/6	5/6
Low Dose				
0.2 ppm Continuous	3/8	0/8	0/8	3/8
1.0 ppm Intermittent[1]	2/6	0/6	0/6	2/6
Control Group	1/8	0/8	0/8	1/8

[1]Current Threshold Limit Value (TLV)

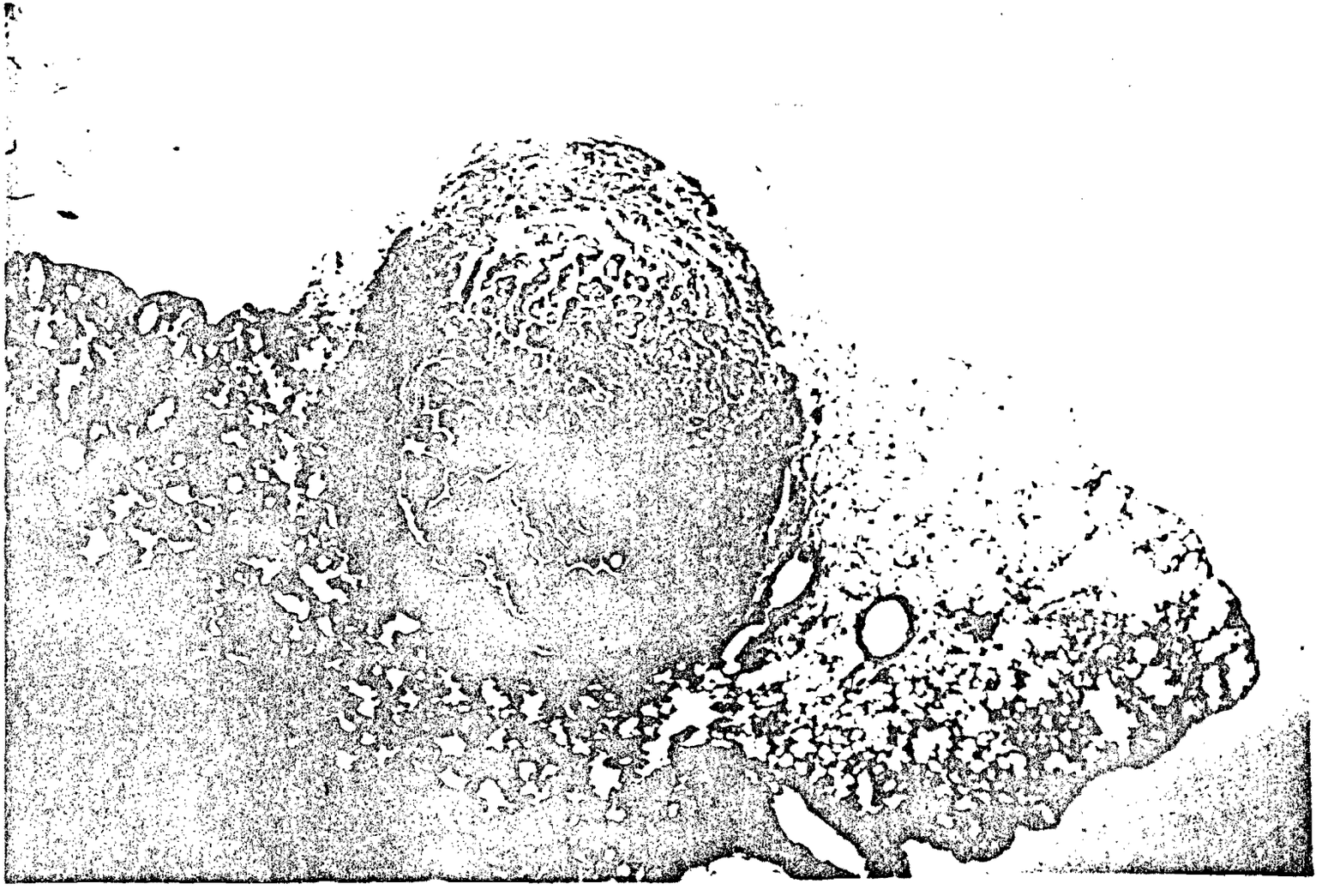

Figure 1. Typical alveolargenic carcinoma found in mouse lung - 8X (1278-74).

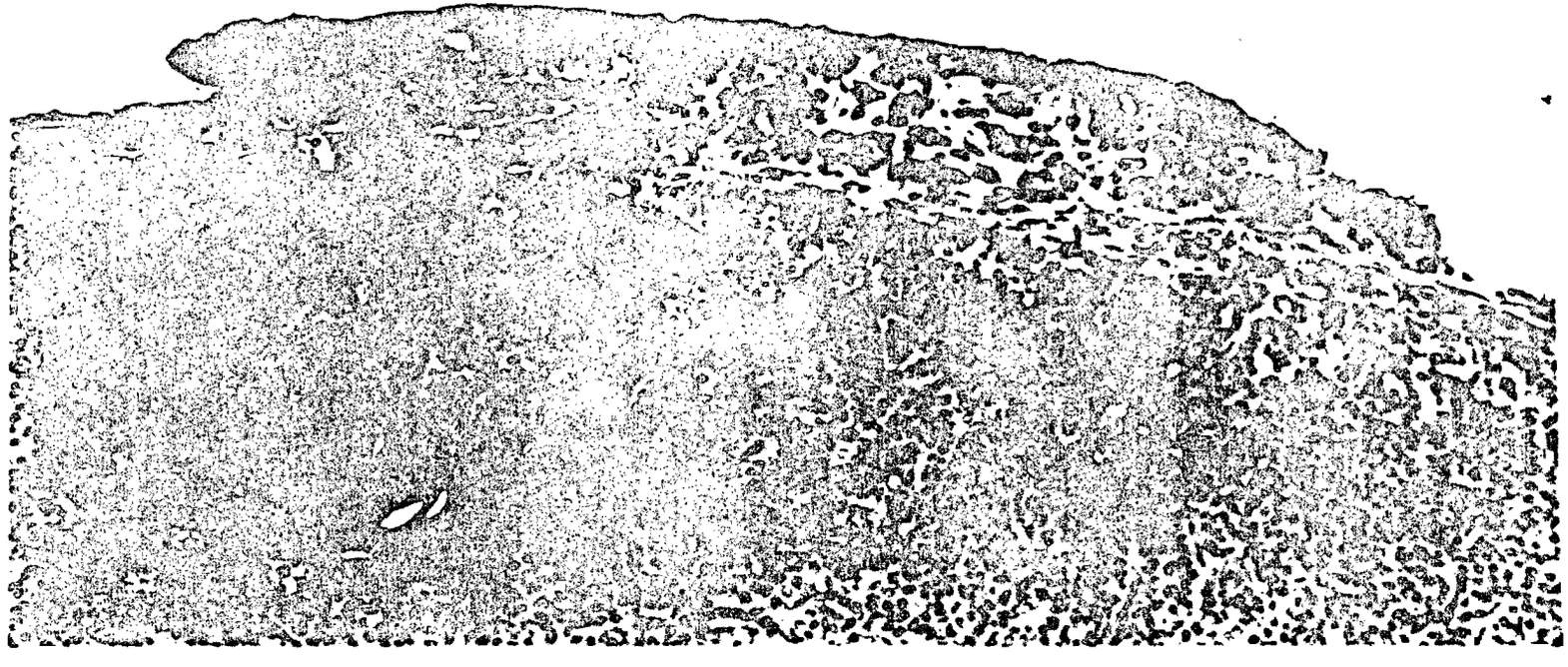

Figure 2. Invasion of pleura by alveolargenic carcinoma - SX (1276-74).

Figure 3. Metastatic lesion of mouse alveolargenic carcinoma in liver - 33X (1271-74).

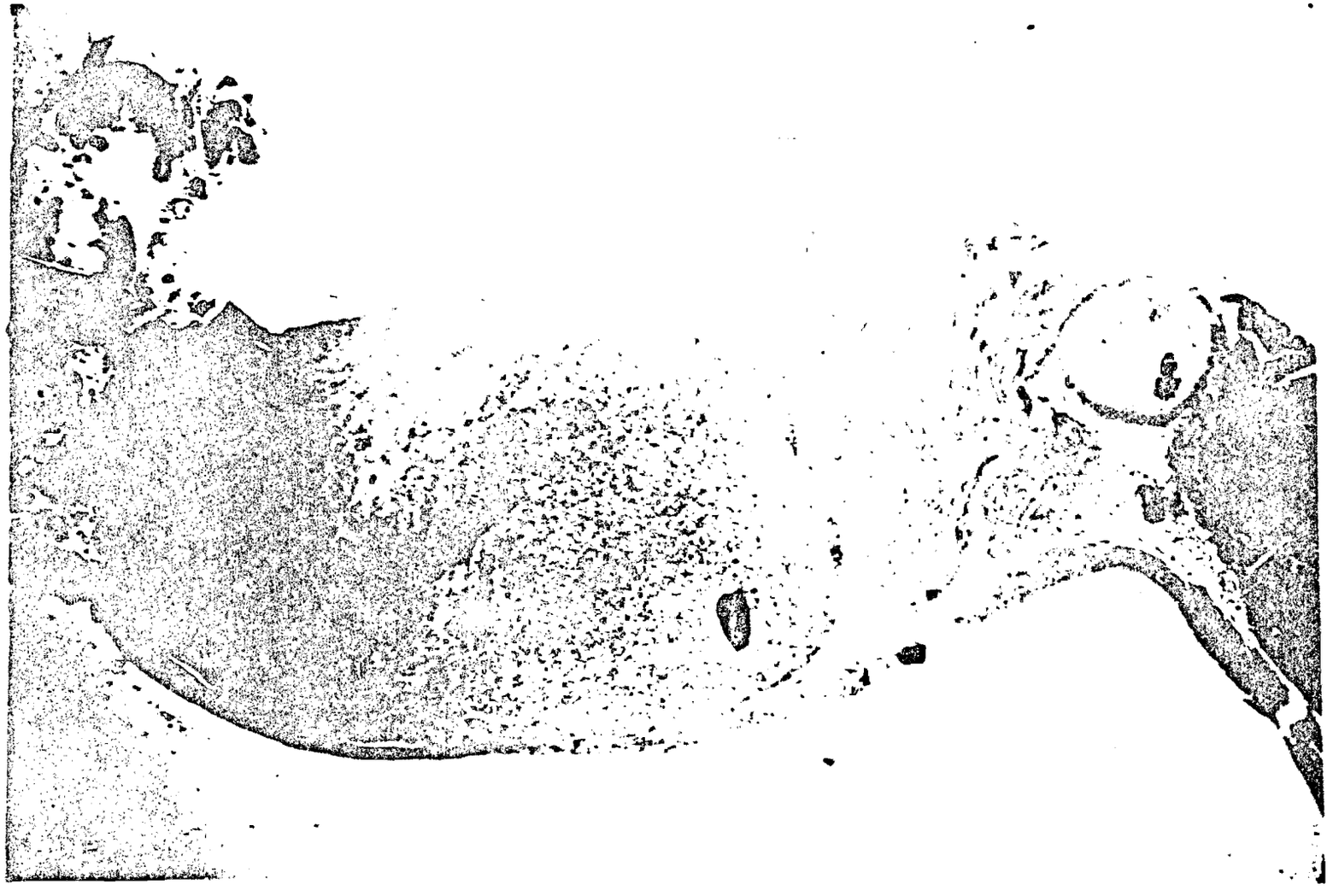

Figure 4. Metastatic lesion of mouse alveolargenic carcinoma in intercostal muscle - 8X (1271-74).

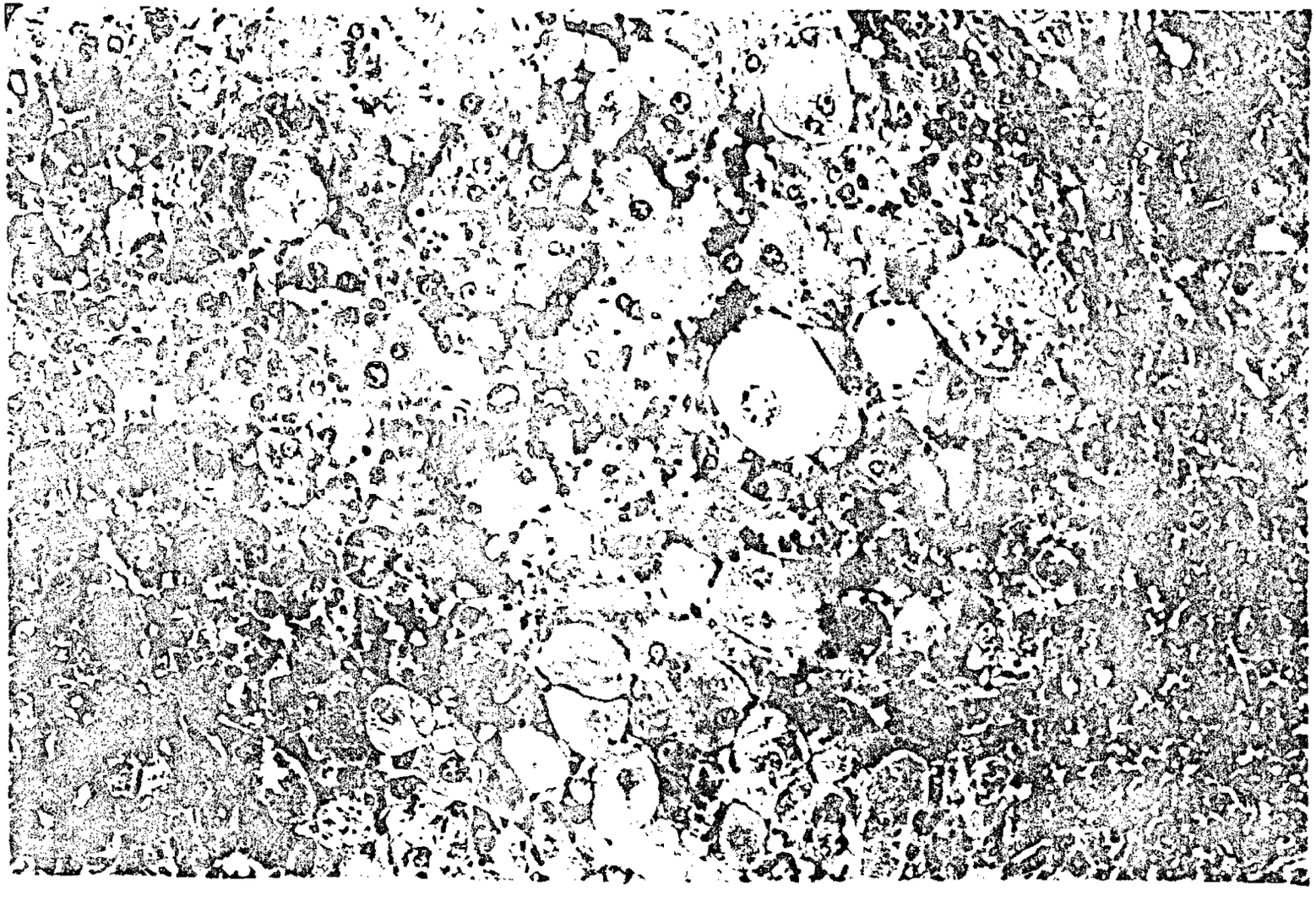

Figure 5. Unorganized liver tissue in mouse hepatoma - 33X (1267-74).

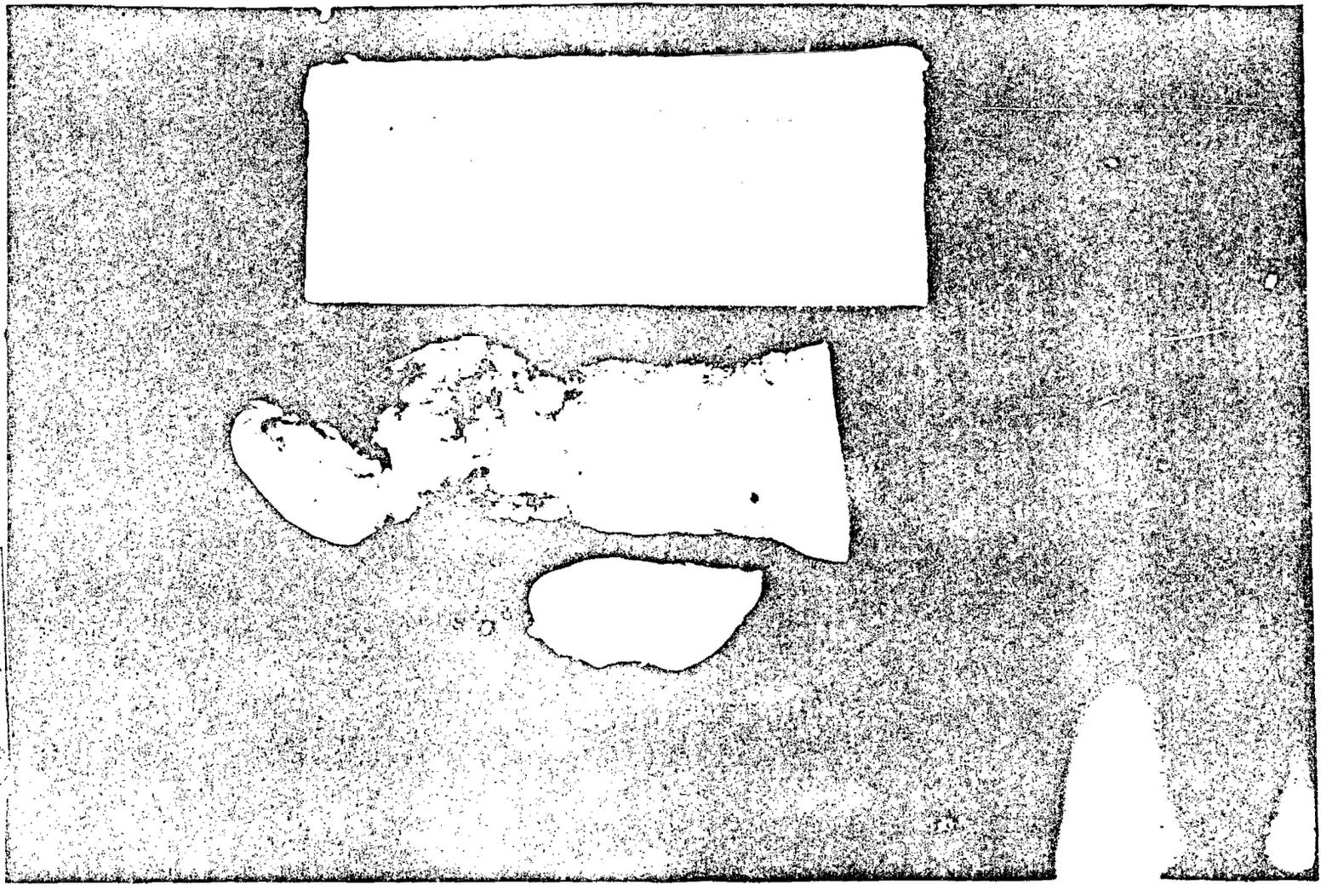

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Figure 6. Splenic hyperplasia in mouse metastatic hepatoma - (1267-74).

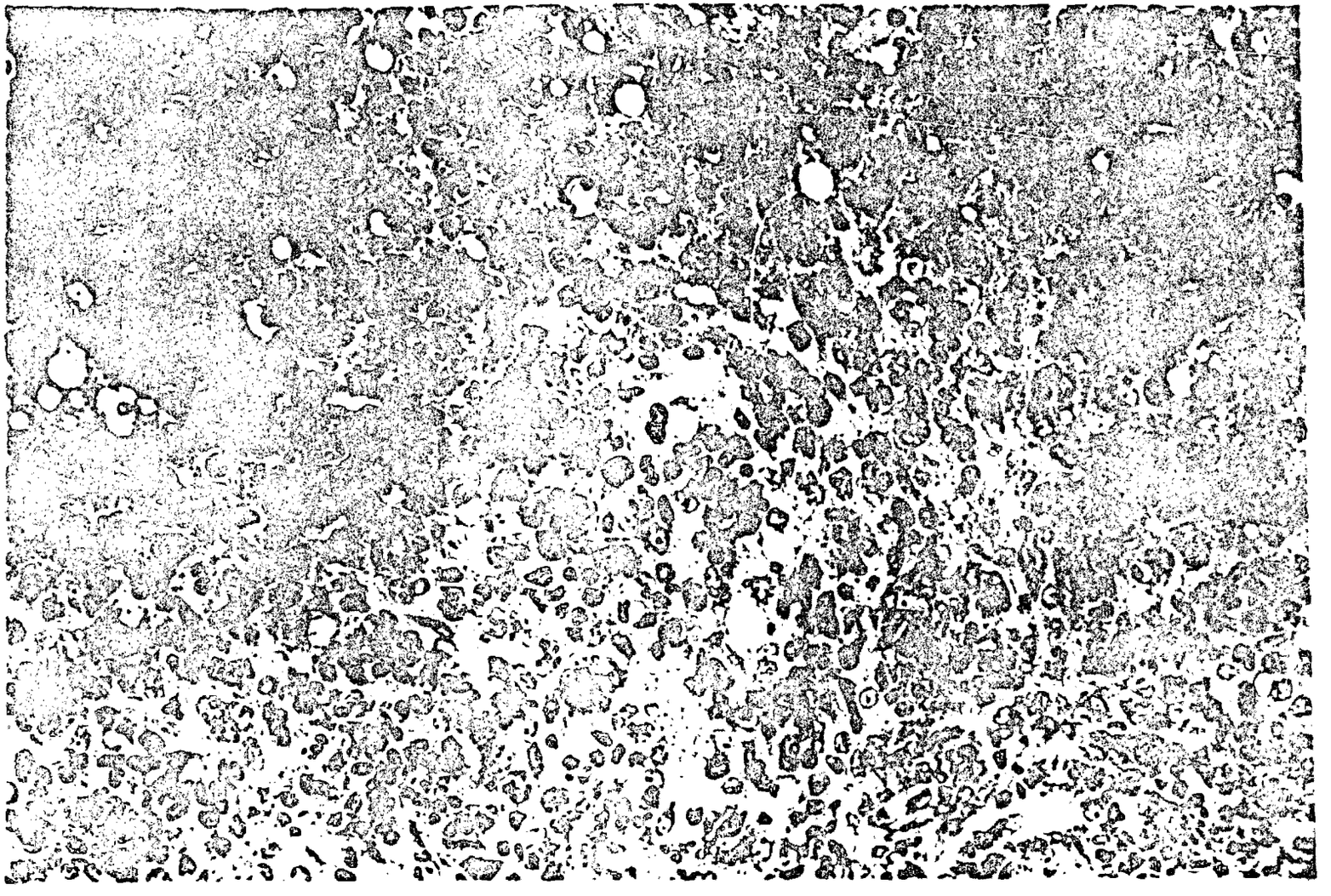

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Figure 7. Splenic neoplasia in mouse hepatoma - 65X (1267-74).

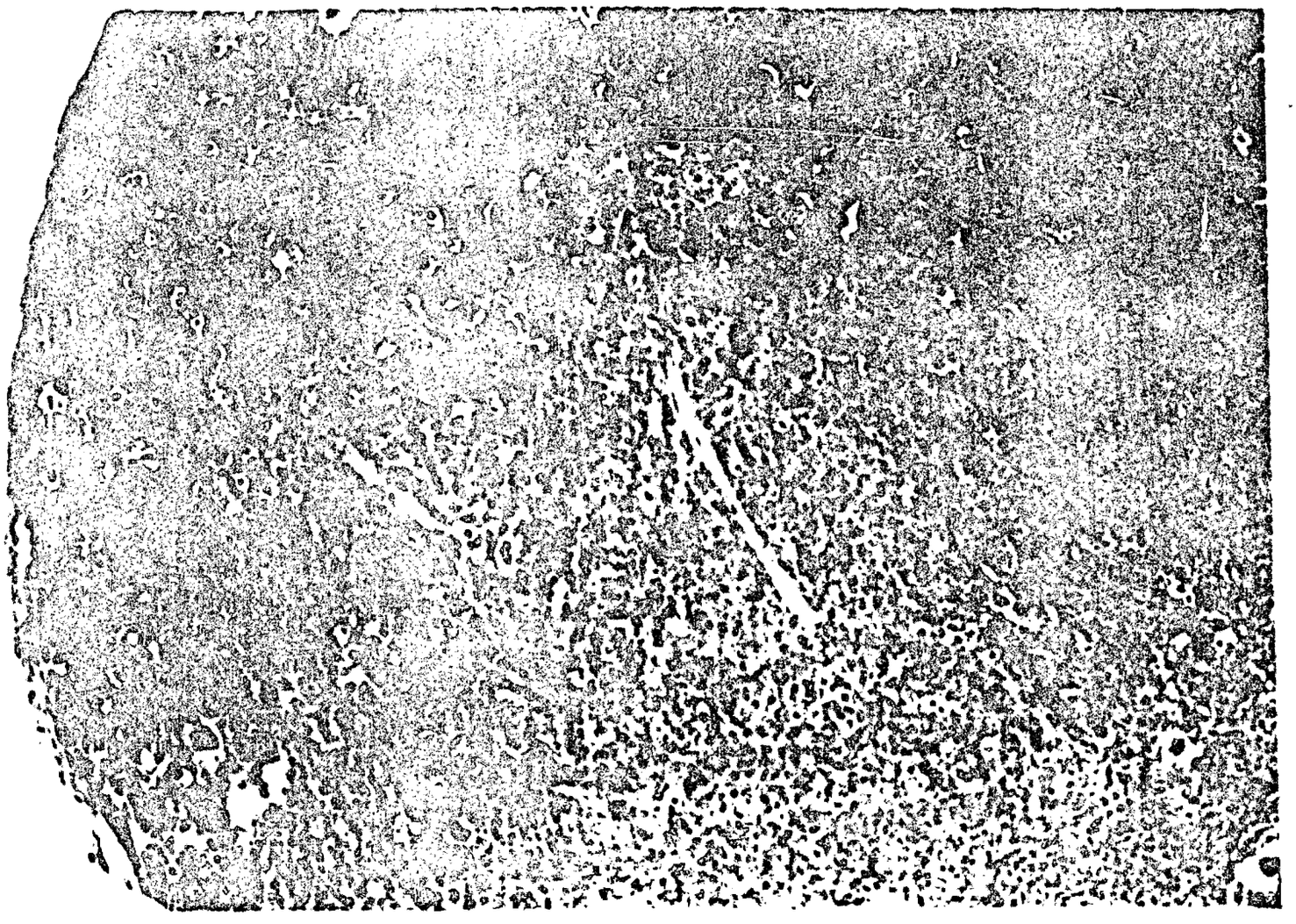

Figure 8. Splenic changes seen with lymphosarcoma in the mouse - 33 X (1265-74).

Figure 9. Neoplastic changes seen in a lymph node with lymphosarcoma - 8X (1265-74).

Alveolargenic carcinomas are "normally" found in older mice with a frequency of about 10% and we have found this rate of tumors in this experiment as well as in other concurrent experiments in this laboratory using the CF-1 strain mice. In previous studies, using electron microscopy, these tumors have been shown to contain Type C virus particles. The virus particle is thought to be the probable etiologic agent for these spontaneous alveolar carcinomas. Another significant finding is the metastatic extension of the hepatoma to the spleen and metastasis of alveolargenic carcinomas to heart and rib cage, respectively, in two other hydrazine exposed mice. These findings are consistent with the induction of lung tumors in mice by hydrazine sulfate reported by Biancifiori et al. (1962a, 1962b, 1963a, 1963b, 1963c, 1966), Biancifiori (1969 and 1970), Roe et al. (1967), and Toth (1969, 1971, 1972). Hepatomas and hepatocarcinomas have also been observed after oral dosing of mice with hydrazine sulfate by Biancifiori (1970a, 1970b, 1970c, 1971) and by Biancifiori et al. (1964). The significance of the findings reported is that this is the first demonstration of hydrazine induced tumors from simulated industrial inhalation exposures at the TLV concentration in mice, albeit in small numbers of animals. These findings should be expanded in additional experiments exposing large numbers of animals of several species to hydrazine by the inhalation route.

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